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PUBLICATION REPORT

1778

12/94



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U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3 (CAIRO, ARAB REPUBLIC OF EGYPT) PSC 452, BOX 5000 FPO AE 09835-0007

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EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS AT A FOCUS MONITORED BY THE MULTINATIONAL FORCE AND ORSERVERS IN THE NORTHEASTERN SINAI DESERT OF EGYPT

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Abstract. A longitudinal epidemiologic study of cutaneous leishmaniasis (CL) transmission was conducted between July 1989 and June 1991 in a 1,200-km² sector of the northeastern Sinai Desert monitored by the Multinational Force and Observers (MFO), an international peace keeping mission between Egypt and Israel. The occurrence of human cases, sand fly density, rodent collection, and isolations of Leishmania confirmed only one of four surveyed locations as a significant focus of CL transmission. Phlebotomus papatasi. the only anthropophilic sand fly species encountered at this focus, comprised more than 96% of the sand fly population and attained human landing densities exceeding 100 sand flies/person/hr during 1990. Seasonal activity of this species ranged from April to November, with highest densities occurring during the period May-September. A peak promastigote infection rate of 2.4% (13 of 534) was observed in P. papatasi during July 1990. Twelve of the 60 (20%) persons at risk during the six months of intense sand fly activity at this site developed lesions consistent with CL; L. major was isolated from nine (75%) of these cases. Leishmania major infection was acquired by two of 22 (9%) sentinel hamsters used during the same period. More than 97% of the 897 wild ro bints trapped at this site were desert gerbil species. Leishmania major was the only Leishmania isolated from human, sand fly, wild rodent (Gerbillus pyramidum), and sentinel hamster infections that originated at site Check point 1-Delta, the focus of CL transmission within jurisdiction of the MFO. The altered ecology of this area, created by construction of a dam, may contribute significantly to the transmission dynamics of CL at this focus.

Cutaneous leishmaniasis (CL) is a disease risk associated with duty in the Sinai-based Multinational Force and Observers (MFO). This selfstyled force was created by the parties to the Camp David Accords, namely Egypt and Israel, with the United States as a witness to the peace treaty. During the first three years of its operation (October 1982-July 1985), 113 of 2,582 members of this peacekeeping mission between Egypt and Israel were diagnosed as having CL.2.1 Leishmania major was the only Leishmania species isolated from these patients. Two other species, L. infantum, which occurs focally in both Egypt and Israel, and L. tropica, which is found widely throughout Israel.4 may also be present in the Sinai. Diagnosis of CL is problematic⁵ and a welltolerated, effective, and simple drug therapy has not yet been developed.6 Leishmania major infections are generally dermatologic and self-limiting, while L. infantum is known to produce mainly visceral pathology (although sometimes

dermatologic) that can be fatal if not treated.⁷ Leishmania tropica also typically produces a self-limiting cutaneous disease, but there is new concern over the viscerotropism and pathology that this parasite has manifested in military personnel that acquired infections while serving in the Middle East during the Persian Gulf War.⁸

At least nine different anthropophilic sand fly species are reported from the Sinai. 10, 11 six of which are either potential or confirmed vectors of leishmaniases. 12 Within the Sinai, the MFO zone of jurisdiction is a broad band extending along the eastern border of the Sinai from the Mediterranean coast to the southern tip of the peninsula on the Red Sea (Figure 1). Within this area of 12,000-km², more than 44 different MFO duty sites are located and during the average yearlong tour of duty, an individual may rotate through 12 different sites. The long incubation period of *Leishmania* infections, variations in the inoculating dose of the parasite, and host

susceptibility make it probable that CL occurrence among the MFO population has been underestimated. Cases may have been missed when the infected individual was transferred before the appearance of clinical lesions, and some inapparent uncomplicated infections may not have been reported at all.

In an effort to assist the MFO in developing a strategy for prevention and control of CL, an epidemiologic study was conducted during 1989–1991 by the U.S. Naval Medical Research Unit No. 3. The objectives of this study were to identify: 1) personnel at risk, 2) temporal and spatial limits of *Leishmania* transmission, 3) *Leishmania* species involved, 4) anthropophilic sand fly species acting as vectors, and 5) rodents serving as zoonotic reservoirs of *Leishmania*. The results of this study are presented here and the risk of acquiring CL is estimated from daily periodicity of sand fly-human contact and monthly measures of sand fly density and infection rate.

SUBJECTS, MATERIALS, AND METHODS

Human component

Clinical records for the Medical Component of the MFO for two years (1983 and 1984) were reviewed to determine past occurrence of CL and to identify risk groups and locations from which CL cases may have originated. New suspected CL cases were identified and case histories were obtained. A sterile biopsy/aspiration was obtained from suspected CL lesions and samples were screened by light microscopy (Giemsastained impression smears) and culture (Tanabe's medium). Tanabe's cultures were monitored for 21 days for growth of Leishmania and positive Leishmania cultures were then transferred to Schneider's medium for rapid growth to stationary phase promastigotes. Promastigotes from stationary phase cultures were harvested, lysed, and identified by enzyme electrophoresis against World Health Organization reference strains according to the methods of Kreutzer and others.13

Sand flies

Sand flies were routinely collected for 5-7 nights each month from four sector 1 locations; El Gorah Main Camp, check point (CP)-1-C, observation point (OP)-1-5, and CP-1-D. They were

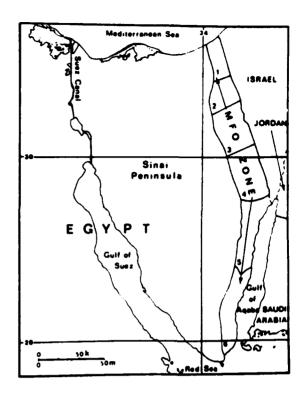


FIGURE 1. Map of the Sinai peninsula showing the Multinational Force and Observers (MFO) zone of jurisdiction and the six sectors of duty assignment. Sectors 1 and 2, Fijian Battalion; sectors 3 and 4, Colombian Battallion; sectors 5 and 6, U.S. Battalion.

randomly trapped (for determining the occurrence of various species in the area) using oiled paper sticky traps and selectively captured by Centers for Disease Control light traps (for collecting species attracted to light) and human landing collections (for catching anthropophilic sand flies). Approximately 8–10 light traps and 20–25 paper traps were placed at fixed locations each night to provide monthly measures of species composition, density, sex ratio, and reproductive age.

Human landing collections were performed at hourly intervals between 8:00 pm and 8:00 AM at CP-1-D. Collectors were equipped with head-lamps, timers, and battery-powered aspirators. Sand flies landing on the exposed arms and lower legs were captured and counted during a recorded time interval in each hour. Mean monthly landing rates were each the result of approximately 10 sampling hours.

Flies obtained in landing collections were held in a cloth cage overnight. The next morning, they were cold-anesthetized, sorted by sex, and females were cryopreserved as described previously. A random sample of each month's cryopreserved collection was thawed and individual females were dissected under sterile conditions to determine species, reproductive status, and the presence and location of promastigote infections. Promastigotes from infected sand flies were aspirated in a 1-cm³ syringe and inoculated into NNN medium and the footpads of BALB/c mice. Leishmania isolates were later transferred to Schneider's medium for growth to stationary phase. They were harvested, lysed, and identified as described previously. 13

Reservoir rodents

Live rodent trapping was conducted for 5-7 nights each month at site CP-1-D in an attempt to identify zoonotic reservoirs of *Leishmania*. Captured rodents were identified by species and sex, examined for suspected *Leishmania* lesions, killed, bled, and necropsied. Samples were taken from all skin lesions, enlarged spleens, and some samples of apparently normal spleens. The samples were screened for *Leishmania* infections by microscopy and for isolation in culture (Tanabe's medium). Positive cultures were treated and identified the same way as described above,

Sentinel hamsters

Golden hamsters (Mesocricetus auratus), recognized as an ideal host for a wide range of sand fly-borne pathogens. 15, 16 were used as sentinels at CP-I-D during the months of sand fly activity. Young (3-4 months old), laboratory-bred animals were close-shaved over their back and sides to provide a large, accessible surface area for sand fly feeding. Groups of four animals were exposed to natural sand fly biting for 5-7 consecutive nights (6:00 PM to 8:00 AM) each month. Exposed animals were returned to the laboratory and monitored for a five-month period for signs of infection. At the end of this period, the animals were killed, bled, and necropsied. Samples of skin and spleen were screened for Leishmania by microscopy and culture (Tanabe's medium). Leishmania isolates were identified as described previously.13

In conducting the research described in this report, the investigators complied with the USDA implementing instructions of the Animal Welfare Act (9 CFR, Subchapter A, Parts 1-3) and

adhered to the Guide for the Care and Use of Laboratory Animals prepared by the Institutes of Laboratory Animal Resources for the National Institutes of Health.

RESULTS

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Identification of risk groups and CL foci

Retrospective analysis of 80 CL cases diagnosed during 1983 and 1984 (Table 1) indicated that Fijian troops, which made up only 19.4% of the MFO population, had accounted for more than 78.7% of the CL cases during that time. The northeastern Sinai (sectors 1 and 2) was identified as the geographic source from which most cases originated and annual incidence within the eight contingents working in this zone ranged from 0.28 to 6.3 CL cases per 100. Estimated incidence was highest among personnel who were assigned to CP or OP duty sites. Within the northeastern Sinai sectors 1 and 2, approximately 3,000-km² in area, Fijian troops are rotated through 16 different duty sites. Comparison of Fijian CL case histories identified three CP and one OP common to most of the cases: CP-1-C, CP-1-D, CP-1-E, and OP-1-5. Sand fly sampling of all these locations during July-December 1989 identified CP-1-D as the only site from which consistently large numbers of sand flies were obtained. Sand fly and rodent collections were thus intensified at CP-1-D during 1990 and personnel on duty at CP-1-D during the study period were prospectively monitored for CL pathology.

Human infections

Diagnoses of CL, based upon the appearance of skin lesions, were made on 16 individuals during 1990. Twelve of these cases (nine of whom were confirmed as CL by the isolation of L. major) were on duty at CP-1-D during the period July-August 1990. Most patients had multiple lesions on the face, arms, or legs, and noted appearance of their sores first in October or November, which was approximately 2-3 months after rotating out of CP-1-D. Based upon these nine confirmed cases of CL among the risk group of 60 individuals who were on duty at CP-1-D during the six-month period of sand fly activity (May-October 1990), a seasonal attack rate of 15% was estimated for the site. Six individuals

Table !				
Cases of cutaneous leishmaniasis by duty location among personnel of the Multinational Force and Observers in				
Sinai, Egypt, 1983–1984				

Location	Contingent	No. at risk/year	No. of cases/two years	Annual incidence/100
Northern Sinai	Civilian	115	2	0.87
	Fijian*	500	63	6.30
	UŠ	350	2	0.28
	New Zealander/Australian	109	1	0.46
	Netherlands	102	1	0.49
	Uruguayan*	75	3	2.00
	French	42	0	0
	U K	37	1	1.35
Central Sinai	entral Sinai Colombian*		6	0.60
Southern Sinai	US*	662	0	0
	Italian	90	1	0.55
		2,582	80	

^{*} Contingents with personnel assigned to check observation point duty sites

who were on duty at CP-1-D during this period, and who may also have been infected with CL, returned to Fiji within two months of their exposure and could not be followed to ascertain the outcome.

Sand flies

Although sand flies were captured at each of the sampling sites (Figure 2), the density of sand flies in paper traps was consistently highest only at CP-1-D. Sand fly activity was detected during the eight-month period from April to November 1990, with greatest densities recorded during July and August. Phlebotomus papatasi was the only anthropophilic species found and made up more than 96.7% of the 8,253 sand flies captured at CP-1-D. The sex ratio of captured members of this species was uneven; females outnumbered males by 2.3:1. Monthly percentages of gravid females, considered to be an index of the physiological age, increased from 13.5% in May to more than 53% in October for the 1990 population captured. Sergentomyia antennata, a reptile feeder, was the only other species found and accounted for no more than 8% of the monthly sand fly population captured.

Human landing collections also detected sand fly activity at CP-1-D during the period April-November 1990. Sand fly landing trends (considered to be equivalent to biting trends) with time and season are depicted in Figure 3. Landing rates were consistently high between 10:00 PM and 4:00 AM and frequently exceeded 100 sand flies/person/hr. The highest hourly landing densities were recorded between 10:00 PM and midnight in the months of May and July. Both paper trap rates and human landing rates showed a sharp decline in sand fly density during the month of June.

Monthly landing rates of sand flies tabulated against dissection results are presented in Table 2. Promastigote infections were found in 0.9% (27 of 2,886) of the P. papatasi that were captured in landing collection during May-October 1990, and the highest monthly infection rate (2.4%) was recorded in July. Leishmania major was isolated from 12 of the 41 infected sand flies (Table 3). The highest percentage of older flies (those with advanced gonotrophic development) occurred in the collections of July (92 of 534 = 17.2%) and October (29 of 162 = 17.9%). A monthly transmission potential for CL, derived from daily duration of sand fly-human contact. monthly human landing density, and promastigote infection rate in sand flies were used to approximate the number of potentially infective sand fly bites an individual would sustain under conditions of unprotected and constant exposure. The highest sand fly landing density and highest promastigote infection rate in sand flies observed in the month of July identified this month of greatest risk for CL infection at CP-1-D.

Reservoir rodents

Rodent trapping, confined to within a 1-km radius of CP-1-D, yielded 897 animals during

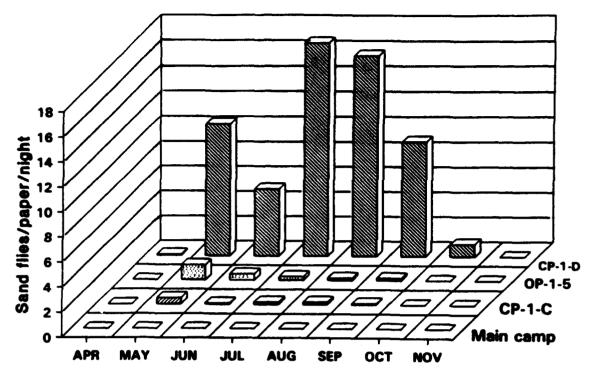


FIGURE 2. Comparative sand fly density/paper trap/night at four locations in Multinational Force and Observers (MFO) sector 1 in northeastern Sinai, Egypt, April-November, 1990.

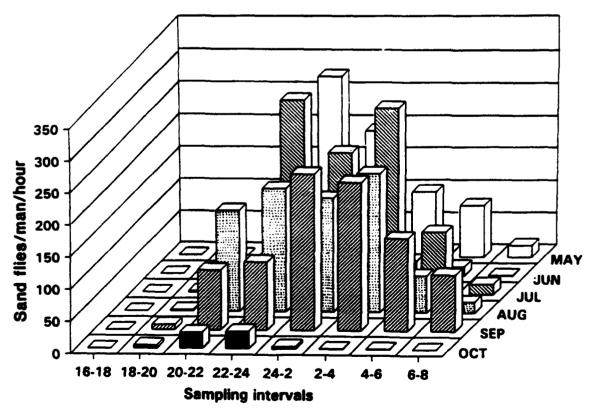


FIGURE 3. Sand fly landing trends with time and season, expressed as sand flies/man/hr at check point-1-D Multinational Force and Observers (MFO) sector 1 in northeastern Sinai, Egypt, May-October, 1990.

TABLE 2

Monthly landing rates, infection rates, and transmission potentials for Phlebotomus papatasi at CP-1-D of the Multinational Force and Observers, Sector 1, in northeastern Sinai, Egypt, April-November 1990

Month	Landing rate/ man/hr	Landing rate/man/ month®	Infection rate, no. positive/ no. tested (%)	мтр
Apr	0.2	60	ND‡	
May	73.1	22,661	0/562 (0)	0
Jun	26.3	7,890	1/566 (0.18)	14
Jul	95.0	29,450	13/534 (2.43)	717
Aug	80.0	24,800	9/517 (1.74)	431
Sep	82.0	24,600	3/545 (0.55)	135
Oct	6.0	1,860	1/162 (0.62)	12
Nov	0.3	98	ND‡	

^{*} Based upon landing rates of 10 hr (8:00 PM to 6:00 AM) of sand fly activity/night.

× (infection rate).

the period from September 1989 to June 1991. Gerbil species (Gerbillus pyramidum, G. gerbillus, and G. andersoni) accounted for more than 95% of the collection (Figure 4). Visual screening of rodents revealed about 1.9% with suspected CL skin lesions, and abnormally large spleens were observed in 12.6% of the rodents. Giemsastained impression smears of skin and spleen biopsy samples of 110 rodents that were suspected of being infected with Leishmania were all negative when examined by microscopy. However, L. major was isolated from one spleen culture (in Tanabe's Medium), and trypanosome epimastigotes were isolated from three of 112 (2.7%) enlarged spleens. Trypanosome parasites have also been subsequently isolated from two of 35 (5.7%) normal spleens. Efforts are currently underway to identify these trypanosomes.

Sentinel hamsters

Multiple cutaneous lesions developed on two of 22 hamsters used as sentinels at CP-1-D during the six-month period of May-October 1990. Leishmania amastigotes were observed by light microscopy in Giemsa-stained skin aspirate preparations and L. major was isolated from the lesions. Both infections had occurred in hamsters that were exposed to sand fly biting during July 1990.

Parasitology

Table 3 outlines the results of Leishmania isolations and identifications obtained during the

TABLE 3
Leishmania isolations from Multinational Force and Observers sector 1 in northeastern Sinai, Egypt

		Identification		
Source	Success- ful iso- lations*	l. major	l pana- mensis	Othert
Human				
Fijian‡	8/11	8		
Colombian	3/4	1	2	
US‡	5/5	5		
Uruguayan	1/1	ı		
Sand fly				
Phlebotomus papatasi	12/41	12		
Rodent				
Gerbillus pyramidum	3/128	1		2

Successful isolations/attempted isolations.

† Unidentified trypanosome.

1989-1991 study period. Leishmania major was the only Leishmania species isolated from human, rodent, and sand fly infections originating at CP-1-D. No variation was observed among the 28 L. major isolates at any of the seven enzyme loci (glutamate-pyruvate transaminase. glucose phosphate isomerase, proline peptidase. malate dehydrogenase, mannose phosphate isomerase, phosphoglucomutase, and phosphogluconate dehydrogenase) that were used for typing these strains. All L. major infections from sand flies produced typical CL lesions within four weeks after inoculation into the footpads of BALB/c mice. Two cases of L. panamensis, isolated from Colombian soldiers, represent recrudescence of infections that had been acquired and

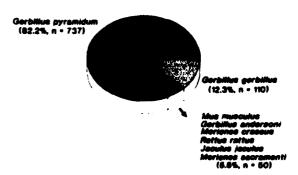


FIGURE 4. Species composition of rodents trapped at check point-1-D Multinational Force and Observers (MFO) sector 1 in northeastern Sinai, Egypt, September 1989-June 1991.

[†] MTP = monthly transmission potential = (landing rate/man/month)

[#] ND = not determined due to the low numbers of sand flies collected.

[‡] All eight Firian and three of five US isolates were obtained from check point 1-D personnel.

treated in Colombia. Unknown trypanosome epimastigotes were repeatedly isolated from the spleens of *G. pyramidum*. Enzyme banding patterns, morphology, and growth characteristics of these organisms are not typical of *Leishmania* and efforts are being made to identify them.

DISCUSSION

Of the four sites studied, the combined results of human case occurrence, sand fly and rodent collection, and Leishmania isolations identify CP-1-D as a principal focus of CL transmission in the northeastern sector of the Sinai monitored by the MFO. Although CL has been recognized in Egypt for nearly a century, this was the first attempt to identify and quantify the components of transmission at a particular focus. This study was unique in its effort to systematically measure vector (sand fly) transmission potential and the risk of acquiring CL.

Although sand fly density, sand fly infection rate, and the human attack rate of CL suggest that CP-1-D was the most important site of CL infection for the MFO population, there is a possibility that CL transmission may be occurring at other MFO duty sites in the Sinai. Simple factors such as lifestyle, clothing preference, and differing tolerance levels to sand fly bites may selectively predispose Fijian personnel to CL infection, while minimizing sand fly contact in other contingents that are assigned duty in or near foci of zoonotic cutaneous leishmaniasis (ZCL). Logistical limitations made it impossible to include 40 other MFO duty sites in the present survey. Extended sand fly surveys using oiled paper traps during the sand fly season (May-October) would help in identifying additional foci of ZCL. There is no reliable information on the occurrence of CL in other parts of the Sinai peninsula, but ZCL foci from the neighboring Negev and Central Arava regions of southern Israel have been reported, several of which are close to the Sinai border. 17-19 The Israeli focus at Keziot, in the Negev, is notable because it is the only other nearby focus that has been well-studied. 19-21 Keziot is situated on the same wadi (dry river bed), less than 20 km from MFO sites OP-1-5 and CP-1-C. However, surprisingly few sand flies were captured during 30 months of standardized survey at these MFO sites and the hypothesis of ZCL in northeastern Sinai as an extension of the Negev focus²² is questioned. Three important

features appear to distinguish the northeastern Sinai focus CP-1-D from other foci of ZCL in Israel: 1) high sand fly density, 2) Gerbillus spp. as the zoonotic reservoir, and 3) sand fly and rodent reservoir Leishmania infection levels.

The phenomenal density of P. papatasi that was routinely recorded by random paper trapping inside this rodent-free check point compound far exceeds any levels that have been reported from endemic foci in Israel. Even among Psammomys colonies in the Jericho focus, paper trap rates averaged only 3.25 flies/paper trap.23 Rates at CP-1-D for the period May-September 1990 were 2-5 times higher than this despite the use of a relatively small paper size. It follows that ecologic conditions must be ideal in this area to produce such enormous populations of P. papatasi. Despite average annual rainfall of only 50-100 mm,24 the watershed created by construction of the El Ruafa dam (1 km from CP-1-D) at the foot of the Gebel Dalfa (428 m) and Gebel Halal (892 m) highlands is possibly responsible for an altered ecology that now generates dense populations of P. papatasi. The heightened water table behind this dam on Wadi El Arish has enabled native desert vegetation and agriculture to flourish, with a concomitant rise in the density of burrowing rodents and their associated arthropods. Anecdotal reports from older members of the local Bedouin community attest to the influence of the dam on sand fly activity. With such high densities of P. papatasi in the MFO zone of operation, there is concern that the New World L. panamensis infections may be acquired by this sand fly vector and established in the Sinai rodent population. Colombian troops, who occupy duty sites in eastcentral Sinai, unknowingly import L. panamensis infections into the MFO each year.23 However, recent studies have indicated that an Indian strain of P. papatasi was refractory to growth of promastigote stage to metacyclic infective stage of L. panamensis and did not support attachment as well as anterior migration of the parasite in the sand fly.26

Gerbillus pyramidum was previously reported as a reservoir host for L. major in northeastern Sinai.²⁷ However, cutaneous lesions and Leishmania isolations were so low among these rodents sampled in the present study that the status of G. pyramidum as a reservoir and the stability of ZCL in the CP-1-D focus is uncertain. Both Psammomys obesus and Meriones crassus, the

principal reservoirs of ZCL in Israel, were conspicuously absent from our collection, as they were from earlier collections made in this area by Morsy and others.²⁷ The presence of mines and unexploded ordnance in northeastern Sinai necessitated cautious sampling for rodents, and was confined to trapping within 1 km of the CP-1-D compound. Possibly a wider search might discover these species in the area. Although Psammomys obesus is rarely taken in traps, it is believed that the specific habitat requirements of this species are not met at this location and its distinctive burrows were not seen. The distribution of Psammomys obesus is apparently limited by a need for saline soil or salt marsh and the succulent holophytic vegetation that grows in such areas. Further indication of its absence from the CP-1-D focus is the preponderance of Gerbillus species in the area. Psammomys obesus has been reported from northeastern Sinai, but is not typically associated with either G. pyramidum or G. gerbillus.24 The nearby Keziot focus is also characterized by a scarcity of Psammomys obesus and abundant M. crassus, nearly 10% of which were found to harbor leishmanial

An apparent discrepancy exists between the Leishmania infection rate reported here for P. papatasi and the occurrence of skin lesions and Leishmania infections in the rodent population sampled. Given the high density of P. papatasi and an average infection rate of approximately 1%, more frequent rodent infections were expected. The fact that a single L. major isolation was obtained from the spleen of a gerbil that had no cutaneous signs of infection suggests that the parasite may produce mild or inapparent pathology in these rodents. In two trials, culture promastigotes of an L. major strain isolated from sand flies collected from CP-1-D were inoculated into the footpads of young, field-collected, unexposed G. pyramidum. Moderate swelling at the site of inoculation was observed four months after inoculation in these animals as opposed to gross swelling in BALB/c controls within 30 days of inoculation (Fryauff D, unpublished data). It is possible that even careful visual screening and necropsy will fail to detect many L. major infections in this host species. It is thus notable that in previous studies, cutaneous lesions with amastigote infections were found in 43% (9 of 21) of Gerbillus spp. captured in an unspecified area of northeastern Sinai.27

Because of the manner in which sand flies were collected, it is inappropriate to compare sand fly infection rates obtained in this study with those that have been reported by previous investigators from this area. Although a single first Egyptian isolation of L. major from P. papatasi in northeastern Sinai has been reported previously,28 the sand fly collections were made by light trap and were not sufficiently standardized to provide useful information on seasonal population trends and promastigote infection rates. Schlein and others have reported high infection rates in P. papatasi,21,23 but flies were captured directly from the burrows of reservoir rodents and may not be representative of the population that carries the infection out of the animal colony and into the human community. In the present study, efforts were thus made to collect those flies and rodents that impinge upon the MFO duty environment. Exacting attempts were also made to standardize sampling methods so that the most accurate profile of sand fly transmission potential and human risk of acquiring CL infection might emerge. It is intended that the profile that did emerge should provide a basis for recognition. prevention, and control of CL in the MFO and stimulate further investigation. In fact, as a result of the present investigation, one such study that involves repellent testing and barrier spray evaluation at selected MFO sites has already been planned and approved.

Acknowledgments: We acknowledge the valuable support and assistance of the Multinational Force and Observers. Special thanks are extended to Capt. R. Carnevale, Maj. R. Phillips, Capt. D. Kumpula, and all members of the Preventive Medicine Section of the United States First Support Battallion and to members of the Fijian Infantry Battallion for assistance and hospitality throughout the study period, Grateful appreciation is extended to Moustafa Osman Ibrahim, Elen Mikhail, Hanafi A. Hanafi, Fetouh Ali Abdel A'Al, and Drs. Michael Wassif and Andrew Main for technical and administrative assistance.

Financial support: This work was supported by the U.S. Naval Medical Research and Development Command, Naval Medical Command, (Bethesda, MD) Work Unit No. 62770A3M162770 AR 322 B.3 and the Multinational Force and Observers (Rome, Italy).

Disclaimer: The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Navy Department, Department of Defense, the U.S. Government, or the Multinational Force and Observers.

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND D	ATES COVERED
i. Addition on the factor of t	1993		
4. TITLE AND SUBTITLE		15.	FUNDING NUMBERS
Epidemiology of Cutaneous Leish	maniasis at a Focus Monitor	ed by	PE- 62770A
the Multinational Force and Obse			WU- 3M162770A870.AR.322
Desert of Egypt			
6. AUTHOR(S)			
Fryauff, David J., Modi, Govind	B., Mansour, Noshy S., Kre	autzer.	
Richard D., Soliman, Sohail and	•		
,			
7. PERFORMING ORGANIZATION NAME	(S) AND ADDRESS(ES)	8.	PERFORMING ORGANIZATION
		[REPORT NUMBER
U.S. Naval Medical Research Un	uit No. 3		
PSC 452, Box 5000			12/94
FPO AE 09835-0007			
9. SPONSORING/MONITORING AGENCY	NAME(S) AND ADDRESS(ES)	10	. SPONSORING / MONITORING
			AGENCY REPORT NUMBER
Naval Medical Research and Dev	elopment	İ	
Command, National Naval Medic	al Center		
Building 1, Tower 12			
Bethesda, MD 20889-5044			
11. SUPPLEMENTARY NOTES			
D 111 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	TT 40/5)-500 (07 1002	A N- 1570	
Published in: Am. J. Trop. Med	. Hyg., <u>49</u> (5):598-607, 1993	; Acc. No. 17/8.	
12a. DISTRIBUTION / AVAILABILITY STAT	EMENT	12	b. DISTRIBUTION CODE
Approved for public release;			
Distribution is unlimited.			
Piperion in minimum.			
13. ABSTRACT (Maximum 200 words)			
·			
A longitudinal epidemiologic study			
June 1991 in a 1,200-km ² sector of		•	
(MFO), an international peace kee			
density, rodent collection, and isol	lations of Leishmania confirm	ed only one of four sur	veyed location as a significant
focus of CL transmission. Phlebo	otomus papatasi, the only ant	hropophilic sand fly spe	cies encountered at this focus,
comprised more than 96% of the s			
flies/person/hr during 1990. Sease			
occurring during the period May-S	<u> </u>		-
papatasi during July 1990. Twelv	· · · · ·		
this site developed lesions consiste			
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14. SUBJECT TERMS	······································		15. NUMBER OF PAGES

OF REPORT

UNCLASSIFIED

VSN 7540-01-280-5500

17. SECURITY CLASSIFICATION

Cutaneous leishmaniasis; Epidemiology; L. major; P. papatasi;

16. SECURITY CLASSIFICATION

UNCLASSIFIED

OF THIS PAGE

Multinational Force and Observers; Sinai desert, Egypt.

20. LIMITATION OF ABSTRACT

16. PRICE CODE

19. SECURITY CLASSIFICATION

UNCLASSIFIED

OF ABSTRACT